Generation of integration-free induced pluripotent stem cell lines from four pediatric ADHD patients

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Lab Resource: Multiple Cell Lines

Generation of integration-free induced pluripotent stem cell lines from four pediatric ADHD patients

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A B S T R A C T

Human induced pluripotent stem cell (iPSC) lines have been derived from four male patients with childhood attention-deficit hyperactivity disorder (ADHD). Children and adolescents between the ages 6 and 18 suffering from ADHD were recruited for this work. Isolated keratinocytes or peripheral blood mononuclear cells from the participants were reprogrammed into iPSCs using non-integrating Sendai virus to deliver the reprogramming factors Oct3/4, Sox2, Klf4 and c-Myc.

Resource Table:

Unique stem cell lines identifier	*TMPi006-A* *TMPi006-B* *TMPi007-A* *TMPi007-B* *TMPi008-A* *TMPi009-A* *TMPi009-B*
Alternative names of stem cell lines	*MR001 c3 (TMPi006-A)* *MR001 c15 (TMPi006-B)* *MR010 c3 (TMPi007-A)* *MR010 c18 (TMPi007-B)* *MR013 c3 (TMPi008-A)* *MR014 c12.1.1 (TMPi009-A)* *MR014 c27 (TMPi009-B)*
Institution	Psychiatric University Hospital Zurich, Department of Child and Adolescent Psychiatry and Psychotherapy, University of Zurich
Contact information of distributor	Prof. Dr. Edna Grünblatt (edna.gruenblatt@kjpd.uzh.ch)
Type of cell lines	iPSC
Origin	Human
Cell Source	Keratinocytes and peripheral blood mononuclear cells
Clonality	Clonal
Method of reprogramming	Sendai virus
Multiline rationale	Control and disease pair (Yde Ohki et al., 2021)
Gene modification	NO
Type of modification	N/A
Associated disease	Attention-Deficit Hyperactivity Disorder (ADHD)
Gene/locus	N/A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	March 2020
Cell line repository/bank	N/A
Ethical approval	Cantonal Ethics Committee (BASEC-Nr.-2016–00101 & BASEC-Nr.-201700825)

1. Resource utility

iPSC lines derived from patients with ADHD are a useful tool to create a model of the disorder. Characterized iPSCs and derived neuronal stem cells or neurons from patients can be compared to healthy control lines to study various molecular pathways and thereby fill the gap of knowledge behind the cellular and molecular origin of ADHD.

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Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease	Primary material
TMPi006-A	MR001 c3	Male	15	Caucasian	N/A	ADHD	Keratinocytes
TMPi006-B	MR001 c15	Male	15	Caucasian	N/A	ADHD	Keratinocytes
TMPi007-A	MR010 c3	Male	9	Caucasian	N/A	ADHD	Keratinocytes
TMPi007-B	MR010 c18	Male	9	Caucasian	N/A	ADHD	Keratinocytes
TMPi008-A	MR013 c3	Male	16	Caucasian	N/A	ADHD	PBMCs
TMPi009-A	MR014 c12.1.1	Male	13	Caucasian	N/A	ADHD	PBMCs
TMPi009-B	MR014 c27	Male	13	Caucasian	N/A	ADHD	PBMCs

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis (Immunocytochemistry)	Expression of pluripotency markers: OCT4, SSEA4, Tra-1–60 and SOX2	Fig. 1 panel A
	Quantitative analysis (qRT-PCR)	Expression of pluripotent genes: NANOG, OCT4, LIN28A, SOX2	Fig. 1 panel E
Genotype	Genetic integrity analysis comparing saliva and iPSC DNA CNVs using genome-wide association array	All lines: 46, XY	Fig. 1 panel C and Supplementary
Identity	Infinium Global Screening Array (Illumina)	N/A N/A	N/A Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing Southern Blot OR WGS	N/A N/A	N/A N/A
Microbiology and virology	Mycoplasma	Negative	Fig. 1 panel B
Differentiation potential	Embryoid body formation	Successful EB generation and expression of ectodermal (SOX2), mesodermal (FLK1) and endodermal (AFP) marker	Fig. 1 panel A and F
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Saliva genotyping HLA tissue typing	DNA analysis N/A	Not shown but available with author N/A

2. Resource details

ADHD is a heterogeneous childhood developmental disorder with a high prevalence of 5% in children and adolescents (American Psychiatric Association, 2013). In the present study, seven iPSC lines were established from four ADHD patients using a Sendai virus reprogramming kit (Invitrogen – Thermo Fisher Scientific) to deliver the four Yamanaka reprogramming factors.

Keratinocytes and peripheral blood mononuclear cells (PBMCs) from ADHD patients were reprogrammed into seven different iPSC lines using Sendai virus (Table 1). The characterization of the derived iPSC lines is summarized in Table 2. All derived clones displayed typical iPSC morphology: The border of the colonies was well defined and the colonies themselves were composed of small cells with prominent nuclei (Fig. 1A, LM: light microscopy, scale bar: 200 μm). The expression of key pluripotency markers OCT4, SSEA4, TRA-1-60 and SOX2 was confirmed in all cell lines by immunocytochemistry proofing their pluripotent potential (Fig. 1A, scale bar: 200 μm). All cell lines were tested for mycoplasma contamination. PCR products generated with a mycoplasma detection kit were run on a 1.2% agarose gel (Fig. 1B). The negative internal control sample showed a distinct control band at 480 base pair indicating a successful PCR reaction. The positive control showed a band at 260 base pair. No mycoplasma contamination was detected. The cell lines had a normal karyotype without any gross genomic aberrations (Fig. 1C represents MR001 c3, see all lines in Supplementary). Sendai virus trace testing was performed using quantitative real-time PCR (qRT-PCR). The positive control, P1 iPSC, showed high expression of SeV (relative expression = 100%) and the negative control, control iPSC, no expression (Fig. 1D). Minimal residues of Sendai virus (relative expression = 0.44%) were detected in cell line MR001 c3 (Fig. 1D). In addition to immunocytochemistry, qRT-PCR was used to reveal the pluripotency potential of the cells. The activation of pluripotent genes such as NANOG, OCT4, LIN28A and SOX2 was confirmed in all cell lines (Fig. 1E). The formation of embryoid bodies (EBs) assessed the differentiation potential of the iPSC lines (Fig. 1A, LM: light microscopy, scale bar: 200 μm). The formation of all three germ layers was confirmed by the expression of endodermal, mesodermal and ectodermal markers using qRT-PCR (Fig. 1F).

3. Materials and methods

3.1. Subject recruitment

Four male children and adolescents with ADHD were recruited by experienced clinicians of the Department of Child and Adolescent Psychiatry and Psychotherapy, University Hospital of Psychiatry Zurich. ADHD of the patients fulfilled the criteria according to DSM-5 as well as ICD-10 (World Health Organization, 1992; American Psychiatric Association, 2013). Standardized clinical interviews have been performed and subjects suffering from a severe psychiatric or neurological comorbidities were excluded to accomplish the utmost homogeneity of the ADHD core symptoms (see Supplementary). Furthermore, the genetic background of each participant was analysed using a polygenic risk score (PRS) analysis. The PRS, a weighted sum of the number of risk alleles a patient carries for a specific disease, was calculated to assess the genetic load using the most recent meta-analysis identifying the first genome-wide significant loci for ADHD association study (GWAS) by Demontis et al. (2019).

3.2. Reprogramming

iPSCs were generated from either patient derived keratinocytes or PBMCs using a Sendai virus CytoTune^{TM}-iPS 2.0 kit (Invitrogen – Thermo Fisher Scientific) carrying three non-integrative viral vectors containing polycistronic Klf4-Oct3/4-Sox2 (KOS), c-Myc and Klf4. Keratinocyte culture and reprogramming was initiated three days prior to viral transduction and the reprogramming was performed in accordance with a previously established protocol (Re et al., 2018). PBMC culture and reprogramming was initiated four days before viral transduction.

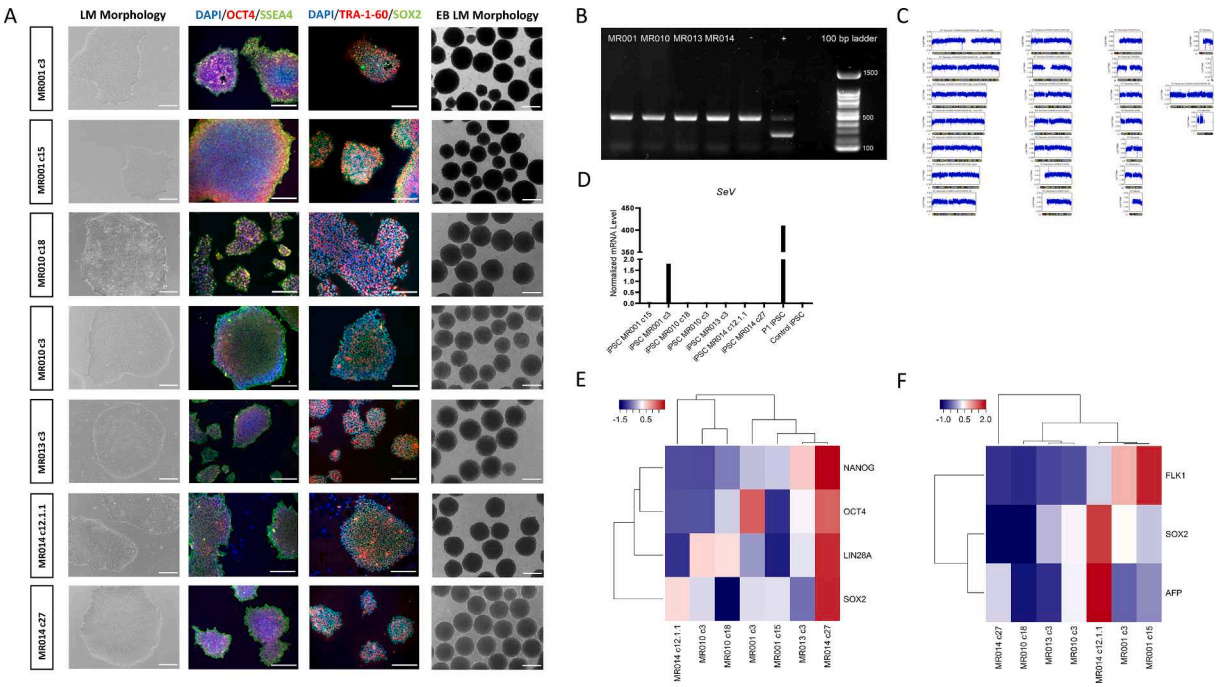

Fig. 1.

The reprogramming was performed according to manufacturer's instructions. Derived iPSCs were cultured under serum- and feeder-free conditions on Vitronectin (Gibco) in E8 medium supplemented with 10 mM Y-27362 (Stemcell™). The iPSCs were passaged every three to four days in a 1:3 splitting ratio using Versene Solution (Gibco). Quality control was performed on all cell lines after reaching passage 11.

3.3. Embryoid body formation

iPSCs were cultured in AggreWells with E8/PVA medium for two days to form EBs according to a protocol by Lin and Chen (2014).

3.4. Immunocytochemistry

Expression of SSEA4, OCT4, SOX2 and TRA-1-60 in iPSCs was assessed with the Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit (Invitrogen™) following manufacturer's instructions (Table 3).

3.5. Real-time quantitative PCR analysis

RNA was extracted from iPSCs using the RNeasy® Plus Mini kit (Qiagen) according to manufacturer's instructions. 500 ng RNA per iPSC sample, respectively 1 μg RNA per EB sample, were reverse transcribed into cDNA using the iScript™ cDNA Synthesis Kit (Bio-Rad) with a C1000™/CFX96™ thermal cycler. Gene expression profiles were assessed using the QuantiFast® SYBR® Green PCR kit (Qiagen). Each sample was run in triplicates. Genes of interest (GOI) and reference genes (RG) were amplified on the CFX384 thermal cycler using the primers detailed in Table 3. LinRegPCR (version 2020.0.0.3) was used to calculate the PCR efficiency and Biogazelle qBasePLUS2 (version 2.3) to normalize the mRNA levels of GOI against the RGs (ACTB and HMBS for iPSCs, ACTB and GAPDH for EBs). A heatmap plot was generated in RStudio (version 1.1.423) with the package heatmap3.

3.6. Detection of SeV genome

iPSC lines were tested for Sendai virus residues by qRT-PCR, as described above, using a primer for *SeV* amplification (Table 3). RNA from a passage one iPSC was used as positive control. Negative control RNA was obtained from a commercial human episomal iPSC line (A18945, Thermo Fisher Scientific, derived from CD34 + cord blood using a three-plasmid, seven-factor EBNA-based episomal system).

3.7. Mycoplasma contamination testing

Absence of mycoplasma was detected by the LookOut Mycoplasma PCR Detection Kit (Sigma-Aldrich – Merck) following manufacturer's instructions. PCR was run on a C1000™/CFX96™ Thermal Cycler. The products were loaded on a 1.2% agarose gel containing HDGreen Plus (INTAS, Germany) and run at 100 V for 30 min. The bands were visualized in the Bio-Rad ChemiDoc™ XRSC System using Image Lab™ (Bio-Rad, version 6.0.0).

3.8. Genotyping analysis

Salivary and iPSC DNA were extracted using the GeneFix™ saliva-Prep DNA kit (Isohelix), respectively the DNeasy® Blood & Tissue kit (Qiagen), and sent for genotyping with Infinium Global Screening Array (GSA, Illumina). The GWAS data by Demontis et al. (2019) was used to assess the PRS of each patient, while genomic alterations were assessed comparing genotypes originating from saliva to the clone's DNA. This was conducted with the Genome Viewer function from the GenomeStudio software (version 2.0).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-OCT4	1:200	Thermo Fisher Scientific Cat# A24867, RRID: AB_2650999
Pluripotency Markers	Mouse IgG3 anti-SSEA4	1:100	Thermo Fisher Scientific Cat# A24866, RRID: AB_2651001
Pluripotency Markers	Rat anti-SOX2	1:100	Thermo Fisher Scientific Cat# A24759, RRID: AB_2651000
Pluripotency Markers	Mouse IgM anti-TRA-1–60	1:100	Thermo Fisher Scientific Cat# A24868, RRID: AB_2651002
Secondary Antibodies	Alexa Fluor™ 555 donkey anti-rabbit	1:250	Thermo Fisher Scientific Cat# A24869, RRID: AB_2651006
Secondary Antibodies	Alexa Fluor™ 488 goat anti-mouse IgG3	1:250	Thermo Fisher Scientific Cat# A24877, RRID: AB_2651008
Secondary Antibodies	Alexa Fluor™ 488 donkey anti-rat	1:250	Thermo Fisher Scientific Cat# A24876, RRID: AB_2651007
Secondary Antibodies	Alexa Fluor™ 555 goat anti-mouse IgM	1:250	Thermo Fisher Scientific Cat# A24871, RRID: AB_2651009

Primers

	Target	**Forward/Reverse primer (5′-3′)**
Pluripotency Markers (qPCR)	*LIN28A*	F: AGCGCAGATCAAAAGGAGACA R: CCTCTCGAAAGTAGGTTGGCT
Pluripotency Markers (qPCR)	*NANOG*	F: TACCTCAGCCTCCAGCAGAT R: CTTCTGCGTCACACCATTGC
Pluripotency Markers (qPCR)	*OCT4*	F: GGAGGAAGCTGACAACAATGAAA R: GGCCTGCACGAGGGTTT
Pluripotency Markers (qPCR)	*SeV*	F: GGATCACTAGGTGATATCGAGC R: ACCAGACAAGAGTTTAAGAGTATGTATC
Pluripotency/ Ectodermal Markers (qPCR)	*SOX2*	F: TGCGAGCGCTGCACAT R: TCATGAGCGTCTTGGTTTTCC
Mesodermal Markers (qPCR)	*FLK1*	F: TGATCGGAAATGACACTGGA R: CACGACTCCATGTTGGTCAC
Endodermal Markers (qPCR)	*AFP*	F: AAATGCGTTTCTCGTTGCTT R: GCCACAGGCCAATAGTTTGT
House-Keeping Genes (qPCR)	*ACTB*	According to manufacturer Qiagen 249,900 (QT00095431)
House-Keeping Genes (qPCR)	*GAPDH*	According to manufacturer Qiagen 249,900 (QT00079247)
House-Keeping Genes (qPCR)	*HMBS*	According to manufacturer Qiagen 249,900 (QT00014462)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102268.

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